#13/A
9019a

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to:
U.S. Patent and Trademark Office, Box Sequence,
P.O. Box 2327, Arlington, VA 22202
on 3 JANUARY 2003.

#35,589

Jeff Lloyd, Patent Attorney

Patent Application
Docket No. USF-T142X
Serial No. 09/832,865

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Examiner	:	(not yet assigned)
Art Unit	:	1633
Applicants	:	Yu Hua
Serial No.	:	09/832,865
Date Filed	:	April 12, 2001
For	:	Regulation of Systemic Immune Responses Utilizing Soluble CD40

U.S. Patent and Trademark Office
Box Sequence, P.O. Box 2327
Arlington, VA 22202

PRELIMINARY AMENDMENT

Sir:

A Petition and Fee for a one-month extension of time through and including January 3, 2003, within which to respond to the Notice to Comply, accompanies this response.

In response to the Notice to Comply mailed October 3, 2002, please amend the above-identified application as follows, in order to comply with the requirements of 37 CFR 1.823:

In the Specification:

Page 6, line 2: Before "Detailed Description of Preferred Embodiments" insert the following:

--Brief Description of the Sequences

A1

SEQ ID NO: 1 is a synthetic nucleotide sequence encoding polypeptide linker.

SEQ ID NO: 2 is a nucleotide sequence of mCD40 forward primer.

A1 Cont

SEQ ID NO: 3 is a nucleotide sequence of mCD40 reverse primer.

SEQ ID NO: 4 is a nucleotide sequence of sCD40 forward primer.

SEQ ID NO: 5 is a nucleotide sequence of sCD40 reverse primer.

Please substitute the following paragraphs:

Page 8, lines 1-6:

A2

The term "fusion protein" denotes the covalent attachment of two or more proteins whereby at least one biological activity of each protein is retained when the fusion protein is expressed. Thus, in a preferred embodiment, sCD40 is expressed as a fusion protein with GM-CSF, in which a linker polypeptide is encoded within the vector in such a manner as to encode an in-frame polypeptide that connects the two proteins. A suitable linker polypeptide is that encoded by the sequence 5'-GCCGCCGCCGCC-3' (SEQ ID NO: 1).

Page 14, lines 2-18:

A3

The cDNA encoding the murine membrane-bound CD40 is obtained by reverse transcriptase-polymerase chain reaction (RT-PCR) of total RNA prepared from spleens of Balb/c mice. Extraction of RNA and RT-PCR is performed as described. A pair of primers is synthesized according to the published sequences and used for amplication of mCD40. The forward primer, 5'-GTC GCT AGC GGG CAG TGT GTT ACG TGC AGT (SEQ ID NO: 2), corresponds to nucleotides 68-89, published in the Journal of Immunology vol. 148, 620-626(2) 1992, which corresponds to a site starting immediately after the putative signal peptide of the mature murine CD40 protein. This primer includes the addition of a 5' Nhel restriction enzyme site and a GTC sequence, the GTC allowing more efficient digestion of the Nhel site. The reverse primer, 5'-CTT GCT AGC ACA GAT GAC ATT AGT CTG ACT (SEQ ID NO: 3), corresponds to nucleotides 546-566 of the gene sequence published in the Journal of Immunology vol. 148, 620-626(2) 1992, which corresponds to a site starting immediately before the transmembrane domain of the mature

A3 Cont murine CD40 protein. This primer includes the addition of a 5' Nhel restriction enzyme site and a CTT sequence, the CTT allowing more efficient digestion of the Nhel site. The final gene product encodes only the extracellular portion of the mature peptide, and excludes the signal peptide, transmembrane and cytoplasmic domains. (Figure 2)

Page 14, lines 19-28:

Amplification of the mCD40 cDNA is performed and the PCR products are purified on a 1.5% agarose gel and directly cloned into the expression vector p at the Nhel sites. The resulting construct, is fully sequenced and no mismatch to published sequence is found. To construct the sCD40 expression vector, another pair of primers is synthesized. A4 The forward primer 5'-GGGCAGTGTTACGTGCAGT-3' (SEQ ID NO: 4), corresponds to nucleotides 71-90, including a site at the beginning of the primer. Nucleotides 9-70 are predicted to encode the leader sequence. The reverse primer, 5'-ACAGATGACATTAGTCTGACT-3' (SEQ ID NO: 5), corresponds to the nucleotides 545-566. The resulting CD40 cDNA portion (71-566) encoding the entire extracellular domain without the leader sequence is cloned into an expression vector. (Figure 1)

Please attach the accompanying Sequence Listing after page 25 (Abstract) as new pages 1-2.